Newborn Screening Facts

Questions Providers Frequently Ask About Newborn Screening

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division of child and Adolescent health

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General Information

Questions Health Care Providers Frequently Ask Regarding Newborn Screening

What diseases in addition to phenylketonuria (PKU) are screened by Virginia Newborn Screening Services? Is the testing mandated?

The *Code of Virginia*, Section 32.1-65, mandates that every infant born in Virginia shall be screened for the following eight diseases of the newborn period: phenylketonuria (PKU), galactosemia, congenital adrenal hyperplasia, hypothyroidism, maple syrup urine disease (MSUD), medium chain acyl coenzyme A dehydrogenase deficiency (MCADD), biotinidase deficiency, homocystinuria, and sickle cell disease and other hemoglobinopathies.

NOTE: "Other" hemoglobinopathies include, for example, C-Disease, thalassemia, and traits/carrier states.

Who is responsible for processing newborn screening samples in Virginia?

Currently, the Virginia Department of General Services, Division of Consolidated Laboratory Services, Newborn Screening Section is responsible for testing all infant samples in Virginia.

How long does the screening process take?

Currently, it takes the Department of General Services, Division of Consolidated Laboratories three days to process and complete the screening on a routine sample. When additional testing is required, the screening time may be lengthened.

When should the newborn screening sample be collected?

According to the Newborn Screening Rules and Regulations, an infant must be equal to or older than 24 hours of age in order to obtain a satisfactory sample for screening**.** Prior to this time, enzyme and amino acid levels may be inadequate and could result in false negative test results. **Furthermore, the sample should be taken prior to administering antibiotics or transfusing blood or blood products.**

What is the cut-off age for an infant when accepting samples for newborn screening?

Samples are accepted from birth to six months of age. Samples taken from children over six months of age are considered unsatisfactory for newborn screening filter paper analysis. The test methodologies employed are set for hematocrit levels ≥ 60%. Any changes in blood saturation, such as those that normally occur with the aging of the infant, may render the test invalid.

What is the difference between confirmation testing and diagnostic testing?

Confirmation testing refers to the screening procedures performed on a second or repeat sample to affirm the results of the initial test. The Newborn Screening Section of the Division of Consolidated Laboratory Services performs confirmatory tests.

Diagnostic testing refers to identification of a disease state or condition utilizing the confirmatory process but also involving other more specialized and specific testing methodologies.

NOTE: Some test methodologies, for example A2 Quantitation, may be used for confirmation or diagnosis depending on the physician's rationale for having the test performed.

Does the Virginia Department of General Services, Division of Consolidated Laboratory Services perform diagnostic studies?

The Division of Consolidated Laboratory Services does not perform diagnostic studies.

What is the difference between an abnormal value and a critical value?

An abnormal value is any test result that falls outside the normal range for that test methodology as established by the screening laboratory.

A critical value is an abnormal test result that may be **highly indicative of a disease process** and, thus, warrants immediate notification to the infant's primary health care provider and the medical consultants by the Newborn Screening Nurse, Virginia Department of Health.

Will I be notified of my patients' newborn screening results?

Results are mailed back to the submitter (usually the hospital of birth) and the primary health care provider listed on the filter paper device for all newborn screening tests. In addition, the health care provider/physician listed on the filter paper device is also notified by telephone within 24 hours regarding all critically abnormal results, by either the Newborn Screening Nurse or by the Newborn Screening Section of the Division of Consolidated Laboratory Services (depending on the presumptive disease).

Whom should I contact for interpretation of laboratory results and for follow-up consultation?

Questions concerning the interpretation of results should be directed to the Virginia Department of General Services, Division of Consolidated Laboratory Services, Newborn Screening Section at (804) 648-4480 ext. 170.

Questions regarding procedures for follow-up should be directed to the Newborn Screening Nurse, Pediatric Screening and Genetic Services, Division of Child and Adolescent Health, Virginia Department of Health (804) 864-7714 or (804) 864-7715.

Is professional medical assistance available if I have an infant with an abnormal result or have questions related to diagnostic testing or treatment protocols?

The Virginia Department of Health has retained the services of metabolic medical and endocrinology consultants to provide assistance with test interpretation, diagnostic testing, and treatment of affected infants. It is strongly recommended that these specialists be consulted to ensure the best outcome for affected or potentially affected infants.

The current metabolic medical consultants for Virginia are:

Arti Pandya, M.D.
Assistant Professor Human Genetics
Virginia Commonwealth University
MCV Campus
1101 E. Marshall St.
PO Box 980033
Richmond, VA 23298-0033
(804) 828-6700
(804) 828-0951 Pager# 3801
Staff: Laura Duncan, M.S., R.D.

William Wilson, M.D.
Professor of Pediatrics
Department of Pediatrics
University of Virginia Health Systems
Box 800386 UVA Health Systems
Charlottesville, VA 22908-0386
(434) 924-2665
(434) 971-6040 Pager
Staff: Barbara Gooding, R.D.

Virginia Proud, M.D.
Professor of Pediatrics
Department of Pediatrics, Division of Medical Genetics
Children's Hospital of the Kings Daughters
601 Children's Lane
Norfolk, VA 23507
(757) 668-9723
(757) 475-5989 Pager
Staff: Melody Persinger R.D.

The current endocrinology consults for Virginia are:

CENTRAL REGION
Anil Kumar, M.D.
VCU School of Medicine
Box 980140, MCV Station
Richmond, VA 23298
(804) 828-9616

NORTHERN REGION
Kathleen M. Link, M.D.
Inova Fairfax and Fairoaks Hospitals
3020 Hamaker Ct., Suite 502
Fairfax, VA 22031
(703) 849-8440

EASTERN REGION
Reuben Rohn, M.D.
Children's Hospital of the King's Daughters
601 Children's Lane
Norfolk, VA 23507-1971
(757) 668-7237

WESTERN REGION
William L. Clarke, M.D.
UVA Medical Center
Box 386
Charlottesville, VA 22908
(434) 924-5897

When is a second newborn screening mandatory?

A second ROUTINE newborn screening test is not mandated in Virginia because it is costly and has not been shown to yield an increase in the number of cases.

The second screen is required **WHEN AN ABNORMAL TEST RESULT OCCURS** or when a sample has been collected when the infant is less than 24 hours of age. When this happens, the submitter (usually the hospital of birth) and the health care provider listed on the filter paper device are notified in writing by the Virginia Department of General Services, Division of Consolidated Laboratory Services, Newborn Screening Section. In cases when a requested repeat specimen is not received by the Division of Consolidated Laboratory Services by 20 work days from the time the initial specimen was received, the Newborn Screening Nurse, Virginia Department of Health places a follow-up call or sends a letter to the submitter or the health care provider listed on the filter paper device requesting submittal of the specimen as soon as possible.

What factors may affect the outcome of the newborn screening tests?

1. Heat
2. Transfusions
3. Antibiotics
4. Foreign substances (e.g., body or tissue fluids, water, detergent, diet, soda, powder from latex gloves, and so forth).

How long should the provider wait to rescreen an infant who has been transfused?

Given the 120-day lifespan of the normal, healthy red blood cell and the presumption that the mean age of red blood cells of the transfused blood is 60-80 days, it is suggested that a sample be collected 60 days after the discontinuance of the transfusion.

When should blood samples be collected from infants who have been or need to be transfused?

As stated in the Virginia Administrative Code, 12VAC5-70-30, "In those instances where the infant requires a transfusion with any blood product, the blood specimen for the newborn screening tests shall be obtained prior to the transfusion. Another specimen shall be taken from the infant immediately upon development of clinical symptoms consistent with a disease specified in the *Code of Virginia*, Section 32.1-65, or at the time of discharge from the hospital, BUT NOT LATER THAN FOURTEEN DAYS OF AGE."

What should we do for an infant who is going to be given antibiotics prior to 24 hours of age?

When it is evident an infant will be started on antibiotic therapy, the blood sample should be collected prior to administration. Samples taken from infants receiving antibiotics at the time of collection may yield abnormal (i.e., false negative) results.

Further, a second sample should be collected immediately upon development of clinical symptoms consistent with a disease specified in the *Code of Virginia*, Section 32.1-65, OR at 24 to 36 hours after discontinuance of antibiotic therapy.

Does acidosis (i.e., metabolic acidosis) affect the newborn screening test?

Metabolic acidosis does not appear to affect the results of the newborn screening test.

Does hypoglycemia affect the outcome of the newborn screening test?

Hypoglycemia in the sick newborn generally should not affect the screening results.

Does total parental nutrition (TPN) affect the outcome of the newborn screening test?

If the infant received total parental nutrition prior to sample collection it may cause false positive results for MSUD, PKU, and homocystinuria,

When should infants born at home be screened?

The *Code of Virginia,* Section 32.1-65, mandates that the physician or certified nurse midwife in charge of the infant's care after the delivery is to collect the blood specimen and submit it to the Newborn Screening Section Laboratory of the Division of Consolidated Laboratory Services, Virginia Department of General Services.

Do I have to pay for the repeat sample, if…
-the repeat is unsolicited by the program?
-the first sample was unsatisfactory as defined in Appendix B?
-the infant was on antibiotics or total parental nutrition (TPN) at the time of the first collection?

Personnel of the Newborn Screening Section of the Virginia Department of General Services, Division of Consolidated Laboratory Services will attach an additional filter paper device when reporting all **abnormal results** which require repeat testing at no additional charge. Any repeat samples taken from infants who received antibiotics, blood transfusions, TPN, or were rejected as unsatisfactory will be tested at the expense of the submitter. **Any other samples not specifically requested which are collected are done so at the expense of the submitter.**

What is the procedure for placing an order for the newborn screening filter paper devices?

Order forms may be obtained by calling the Virginia Department of General Services, Division of Consolidated Laboratories at (804) 648-4480 ext.103 or toll free at 866-378-7730. The forms are to be completed and returned, together with a check for the amount of the purchase, to the Virginia Department of General Services, Office of Fiscal Services. Kit orders are processed upon receipt of the prepaid offer. Devices can also be ordered by phone when a credit card is used. The number to call for credit card orders is 804-225-3345.

Can my patient be referred to the local health department if repeat testing is required? Is there a cost involved?

There is no cost involved **when a repeat test is required by Virginia Newborn Screening Services and a second filter paper card will accompany the abnormal results.** If a **serum test** is requested, the responsibility rests with the primary care physician and/or patient's parent or guardian to make financial arrangements for direct or third party payment to the laboratory performing the testing. Referral to the local health department is not recommended.

How can a copy of the infant's newborn screening results be obtained?

To get a copy of newborn screening results, write to:

Virginia Department of General Services
Division of Consolidated Laboratories
600 North 5th Street
Richmond, VA 23219
ATTN: Newborn Screening Laboratory
Phone: (804) 864-4480 ext. 170 FAX: (804) 225-2595

Please provide information regarding the infant's name, date of birth, mother's name, mother's social security number, hospital of birth, and sample number if known to assist in identifying the appropriate records.

How can a copy of the current Newborn Screening Rules and Regulations be obtained?

Copies of the Newborn Screening Rules and Regulations are available upon request by contacting the Newborn Screening Nurse, Pediatric Screening and Genetic Services, Division of Child and Adolescent Health, Virginia Department of Health at (804) 864-7714/7715 or FAX to (804) 864-7721. They are also available on the Virginia General Assembly website (http://legis.state.va.us), Statutes and Regulations, Virginia Administrative Code, 12VAC5-70; Sections 10-50.

Disease-Specific Information

Disease-Specific Information and Confirmatory Testing

When a serum sample for hypothyroidism is required, where should the specimen be sent for testing?

Any **serum sample** for hypothyroidism should be sent to a hospital or private laboratory for testing.

Serum samples for other disorders may be requested for confirmation or diagnostics by the medical consultants. The medical consultant will provide specific information pertaining to collection and to which laboratory/agency it is to be sent.

NOTE: The Newborn Screening Section of the Division of Consolidated Laboratory Services serves as Virginia's **screening** laboratory and, therefore, does not process clinical samples for diagnostic studies.

How should repeat screening of the premature infant with an initial test result of low T4 and TSH within normal limits be handled?

When the premature infant has a birth weight less than three pounds (1.364kg), TSH is within normal limits, and a low T4, the provider should repeat the newborn screening test at eight weeks of age OR four pounds (1.820kg)--whichever comes first. If the repeat test results indicate low T4, it is recommended that a serum sample be collected for a thyroid profile and TBG level.

TBG deficiency is a congenital shortage of thyroxin-binding globulin, a serum glycoprotein that binds tightly to thyroid hormones and provides a way of storing these hormones in the circulation, releasing them slowly as the tissues need thyroid hormones. TGB deficiency primarily affects males, has a frequency of about 1:10,000, and is suspected when a healthy infant has persistently low T4 values (less than 5 ug/dl) with a normal TSH level. The diagnosis can be confirmed by direct measurement of the TBG level in serum. Since the free or active T4 level is normal, no follow-up or treatment is necessary.

What is FAV? Is this a sickle cell disorder? What is the treatment protocol?

FAV stands for Fetal Hgb-Adult-Hgb plus a Variant. There are 400 + structurally different hemoglobin types identified to date. The majority constitutes a single amino acid replacement in one of the globulin polypeptide chains. Most screening laboratories have the capability to identify seven to ten hemoglobin types. The remaining hemoglobin types are then designated as variants. Variants rarely cause problems or require repeat routine or specialized testing or treatments. Variant ("V") IS NOT a type of hemoglobin. It is a term used to denote the remaining 400+ hemoglobins not specifically named by the lab apparatus. If there is a desire to identify the specific hemoglobin band, it is recommended that a specialized laboratory be contacted to determine precisely what type of blood specimen is required for the testing and, also, that the parents' blood be analyzed to determine the source of the variation.

What is FAB?

FAB stands for Fetal Hgb-Adult Hgb-Barts Hgb. Hgb Barts normally disappears within 6 months of age. However, identifying Barts at birth can assist with identification of Alpha Thalassemia. If Hgb Barts is observed during newborn screening, it will be reported to the primary care provider. Follow up of Barts is left to the discretion of the primary care provider. No further testing is required by the Virginia Newborn Screening Services.

Is Barts Hgb clinically significant?

Less than 5% Barts indicates that the baby may be a silent carrier of Alpha Thalassemia and should have no clinical problems. From 5% to 10% Barts indicates that the baby will probably have a mild anemia that will not be cured by taking iron. There should be no other clinical problems. More than 10% Barts indicates the need for further medical evaluation. Division of Consolidated Laboratory Services Screening/Methodology does not provide results in percent. Some physicians order hemoglobin electrophoresis after six months of age to ensure that Barts has disappeared.

What is Thalassemia? How does it differ from sickle cell disease?

Thalassemia is any one of a group of inherited autosomal recessive blood disorders in which there is failure in the synthesis of one of the globin chains, resulting in an anemic state and ineffective erythropoiesis. The typical thalassemia red blood cell has lower than normal amounts of Hgb A, is microcytic, hypochronic, and may take a banana, sickle or other configuration. The thalassemias occur more frequently in populations from countries bordering the Mediterranean, and Southeast Asia, India, and Southern Europe.

When a presumptive positive for thalassemia is received, it is recommended that a Quantitative Hgb A2 test be performed. The mean corpuscular volume (MCV) and the amount of A2 in the hemoglobin is needed in order to identify the type of thalassemia present.

NOTE: A persistently depressed Hgb with a normal erythrocyte protoporphyrin is often suggestive of Thalassemia Minor. Performing an A2 Quantitation should also be considered.

What are the screening and confirmatory tests for sickle cell disease?

The **screening test** for sickle cell disease uses an electrophoresis technique known as isoelectric focusing, which causes separation of the different hemoglobin bands. The bands are then compared to a control to determine their identity. The **confirmatory test** for hemoglobinopathies, High Pressure Liquid Chromatography (HPLC), gives the quantitative values and positively identifies the Hgb band.

Should Sickle-Dex be used to confirm the diagnosis of sickle cell disease?

SICKLE-DEX AND OTHER SOLUBILITY TESTS SHOULD NOT BE USED TO IDENTIFY SICKLE CELL DISEASE IN INFANTS. They lack sensitivity and specificity in the first few months of life. The amount of fetal Hgb in newborns can mask the S Hgb and give a false normal result. They lack the ability to identify Hgb C or SC Disease.

Laboratory Test Methodologies

Disease-Specific Testing Procedures

I. The technology used to test for **phenylketonuria (PKU), maple syrup urine disease (MSUD), and homocystinuria (HCU)** is Tandem Mass Spectrometry. This testing produces ions from the compounds in the sample and analyzes the fragments according to mass. The quantities of amino acids such as phenylalanine, leucine or methionine are measured against internal standards for these substances.

II. **Congenital hypothyroidism** is detected by immunofluorescent IFA techniques for thyroxine (T4) and thyroid stimulating hormones (TSH). IFA first measures the level of T4 in the blood. For infants whose T4 level falls in the lowest 10% of the results for the assay, TSH is measured on the same specimen. An elevated level of TSH indicates primary hypothyroidism, and the responsible physician is directed to have confirmatory T4 and TSH tests performed on a sample of the infant's serum. These methods are highly specific and sensitive. Testing procedures (IFA) require 5 hours.

III. The test procedure for **galactosemia** is the Beutler test that is a biochemical assay detecting galactose-1-phosphate uridyl transferase enzyme. Specimens which demonstrate decreased fluorescence on the Beutler test receive the Hill test to quantitate the amount of galactose as well as galactose-one-phosphate present in the blood. The Beutler procedure involves a 4-hour process, and the Hill procedure requires an additional 4 hours.

IV. **Biotinidase deficiency** is detected by a colometric test procedure. After incubation and development, a purple color indicates the presence of adequate biotinidase enzyme activity. The absence of color indicates very low biotinidase enzyme activity. Testing procedures require 24 hours.

V. **Newborn hemoglobinopathies** are screened using an electrophoresis technique known as isoelectric focusing. This procedure causes the separation of different types of hemoglobin within a blood sample. Each abnormal hemoglobin band is read against a known control to determine its suspected identity. Testing procedures require 8 hours.

The confirmation test procedure for hemoglobinopathies is performed on a High Pressure Liquid Chromatography (HPLC) and is a 1-hour procedure that gives quantitative values and positive identification of the observed hemoglobin bands in the blood spot sample. This process allows for a final screening result using a filter paper sample, thereby eliminating a requirement for a whole blood sample.

VI. **Congenital adrenal hyperplasia (CAH)** is detected by an immunofluorescent IFA technique for 17-hydroxy progesterone (17-OHP). IFA first measures the level of 17-OHP in the blood. For infants whose 17-OHP is in either the lowest 3% of results or below 40 ng/mL, the test result is confirmed by repeat testing. The interpretation of the generated 17-OHP result is then based on the infant's birth weight. Testing procedures (IFA) method is highly specific and sensitive and requires 5 hours.

VII. **Medium Chain Acyl Coenzyme A Dehydrogenase Deficiency (MCADD)** is detected by using the technology of Tandem Mass Spectrometry (MS/MS). Tandem Mass Spectrometry measures the level of octanoylcarnitine, a product of fatty acid metabolism, that accumulates if the MCAD enzyme is deficient.

Disease Descriptions

Disease Incidence Rates and Characteristics

Phenylketonuria
Hypothyroidism
Galactosemia
Maple syrup urine disease
Homocystinuria
Biotinidase deficiency
Sickle cell disease and other hemoglobinopathies
Congenital adrenal hyperplasia
Medium Chain Acyl Coenzyme A Dehydrogenase Deficiency

I. **Phenylketonuria (PKU)** – national incidence rate 1:20,000

A defect in the metabolism of the essential amino acid phenylalanine results in excessive accumulation of phenylalanine and abnormal metabolites in body fluids of PKU patients. Although the mechanism by which phenylalanine or its metabolites cause brain damage is not clear, limiting phenylalanine in the diet to maintain normal or nearly normal serum phenylalanine levels prevents mental impairment. **Untreated phenylkentouria causes severe mental retardation**. Few clinical signs arouse suspicion of PKU infants. There may be vomiting, feeding dificulties, pyloric stenosis, excessive irritability, or excessive sleepiness. The baby usually is physically normal and progresses normally for the first months of life, but developmental milestones, such as sitting up and turning over, may be delayed. Without treatment, retardation progresses.

II. **Hypothyroidism** – national incidence rate 1:4,000

Congenital hypothyroidism is one of the most common and treatable causes of mental retardation. It usually is due to an absent or abnormally developed thyroid gland. Clinical signs and symptoms in affected infants include edema, dry or mottled skin, poor feeding, poor muscle tone, and decreased activity. Unfortunately, these signs and symptoms may take months to become apparent. The mental deficiency resulting from this condition can be prevented by early diagnosis and treatment with thyroid hormones, preferably before one month of age.

III. **Galactosemia** – national incidence rate of 1:60,000

Classic galactosemia is an inherited defect of carbohydrate metabolism in which glactose cannot be converted to glucose because of missing or deficient enzymes. The disease has severe consequences for infants who are on a milk diet, since galactose is a component of the milk sugar, lactose**. The galactosemic infants may appear normal at birth, but other symptoms appear within a few days, such as jaundice, hepatomegaly, cataracts, hypoglycemia, feeding difficulties, coagulation problems, and decreased immunity**. Without treatment, infants often die of E. Coli sepsis. Those who survive the liver disease and hemorrhagic episodes have cataracts and are physically and menatlly retarted. The treatment of choice is a galactose-free diet.

IV. **Maple syrup urine disease (MSUD)** – national incidence rate 1:100,000

MSUD results from a defect in branched-chain amino acid metabolism. This disorder was so named because of an unusual odor characteristic of maple syrup or burned sugar that appears in the urine, breath, and skin of affected infants. It is caused by the accumulation of amino acids and their corresponding ketoacids in blood, urine, and cerebral spinal fluid secondary to the blocking of oxidative decarboxylation of leucine, isoleucine, and valine. Affected infants are normal at birth, but during the first days of life, they

become listless, refuse to eat, and vomit. This state progresses to loss of reflexes, alternating hypertonicity and hypotonicity, convulsions, and irregular respiration. **Untreated infants are first lethargic, then comatose**. They may die of respiratory disturbances unless diagnosed promptly. Those who survive the acute neonatal period show severe mental retardation and cerebral palsy. There have been no reports of pregnancy in women with the disease. The treatment of choice is restricted branched-chain amino acid intake through diet.

V. **Homocystinuria** – national incidence rate 1:200,000

This disease results from a deficiency of cystathionine synthetase, which normally converts methionine to cystine. Consequently, homocystine and its precursor, methionine, accumulate in blood and urine. **Infants have no clinical symptoms, but they appear later involving the connective tissues, central nervous system, and cardiovascular system**. Lens ectopia is typical, and may cause glaucoma, myopia, retinal detachment, and cataracts. The skeletal system consistently shows genu vulgum (knock knee) with frequent chest, vertebral, and foot deformities. Major motor seizures are often present. The deadly complication is in the cardiovascular system, where multiple arterial and venous thromboses occur as a result of enhanced platelet stickiness. Mental retardation is a common but infrequent finding and probably results from vascular occlusive disease. The treatment of choice is either a pharmacological dose of pyridoxine or, for pyridoxine-resistant patients, a low methionine diet supplemented with cystine.

VI. **Biotinidase deficiency** – national incidence rate estimated to be 1:70,000 (based on 1,000,000 babies screened)

The enzyme biotindase catalyzes the removal of biotin from biocytin or from biotinylated peptides, thereby releasing free biotin for recycling by the body. Children with biotinidase deficiency usually begin to show symptoms at several months of age**. If untreated, they develop a variety of cutaneous or neurological abnormalities, ketosis, seizures, permanent mental impairements, coma, or death**. Affected children may have cutaneous or neurological features without metabolic acidosis or detectable organic aciduria. Therefore, definitive diagnosis requires the demonstration of the enzyme deficiency. The treatment of affected children with pharmacologic doses of biotin results in rapid clinical improvement, and if initiated sufficiently early, can reverse most of the symptoms and prevent their recurrence.

VII. **Sickle cell disease** – national incidence rate 1:400 (African-Americans)

Sickle cell disease is a hereditary disorder involving the protein portion of the hemoglobin molecule. Hemoglobin S is produced when the amino acid valine is substituted for glutamic acid on the beta chain. This disorder predominates in persons of African ancestry but is also diagnosed in persons of Middle Eastern and Indian ancestry. The first clinical indicators, visible by 10 weeks of age, show reticulocytosis, sickling of red cells, and uncompensated hemolysis. Increased Pneumococcal, Salmonella, and Hemophilus influenza infections are of major concern. **Pneumococcal pneumonia and meningitis can lead to the rapid decline or death of these children**. Sickle cell children are often jaundiced and demonstrate enlarged spleens in early childhood. Painful crises may occur in the extremities and abdomen. Secondary to the anemia, the heart is often dilated and flow murmurs are heard. Although there is no cure for the disease, early diagnosis and penicillin prophylaxis against infection can greatly improve the well being of the infant who has sickle cell disease.

VIII. **Congenital adrenal hyperplasia (CAH)** – national incidence rate 1:10,000 to 1:15,000

CAH is a family of inherited disorders affecting the adrenal glands. CAH occurs when a baby's body cannot produce any or enough of certain essential hormones cortisol and aldosterone. These hormones are needed for the body to:

- maintain blood sugar levels
- retain sodium for electrolyte balance
- enhance normal growth and development.

In its most severe form, "classic" CAH, an infant may experience a life threatening adrenal crisis within weeks of birth. **If unidentified and untreated, death may occur**. Milder forms of CAH are not life threatening and may not be identified until early childhood. The most common cause of CAH is the missing enzyme 21-hydroxylase. Screening for CAH measures the level of 17-hydroxyprogesterone (17-OHP), a precursor of cortisol in the dried filter paper blood spot. Treatment is aimed at replacing the missing hormones to promote normal growth and body function. Persons with CAH must take the hormones for life.

NOTE: Because normal values are based on the infant's weight, it is very important to accurately document the weight along with the gestational age and the health status (normal or sick) on the filter paper collection device.

IX. **Medium Chain Acyl Coenzyme A Dehydrogenase Deficiency (MCADD)** – national incidence rate 1: 6,500 to 1: 20,000 live births (CDC).

MCADD occurs when a baby's body cannot produce an enzyme necessary to convert fat to energy during times of decreased food intake or fasting. It is the most common inherited error of fatty acid metabolism. **When infants with MCADD do fast, they can experience a range of serious life threatening symptoms or even death.**

With early identification, treatment for MCADD is fairly simple and allows for a good prognosis. Treatment consists of avoiding fasting for more than 2 to 3 hours in an infant. A high carbohydrate diet with restriction of fats and supplemental L-Carnitine is sometimes recommended, especially during times of illness.

Appendices

APPENDIX A

Normal, Abnormal and Critical Test Values

NBS Laboratory Tests Ranges

A. Normal Ranges (Within Normal Limits)

1. Phenylketonuria: Initial Phenylalanine level < 242 µmol/l
2. Homocystinuria: Initial Methionine level < 134 µmol/l
3. Maple syrup urine disease: Initial Leucine level < 305 µmol/l
4. Galactosemia: Initial Beutler - enzyme activity present; Hill < 10 mg/dl
5. Biotinidase deficiency: Initial level - enzyme activity present
6. Hypothyroidism
   a) T4 > 5.5 mcg/dL (standard cut-off value)
   b) TSH = 0 to 24 µU/ml
7. Hemoglobinopathies
   a) (F/A) Fetal and adult hemoglobin present with fetal hemoglobin in predominance.
   b) (A/F) Adult hemoglobin present in slightly higher quantity than fetal hemoglobin for an infant that has not been transfused. (Seen commonly in babies 2 months or older.)
8. Congenital adrenal hyperplasia

Birth weight category	Normal 17-OHP (ng/ml)
< 1250 gms	<135
1250-1749 gms	<90
1750-2249 gms	<65
≥ 2250 gms	<50

9. Medium chain acyl coenzyme A dehydrogenase deficiency: Initial Octanoylcarnitine level of < 0.5 µmol/l.

B. Abnormal Ranges

1. Phenylketonuria: Initial Phenylalanine level ≥ 4 - < 10mg/dl
2. Homocystinuria: Initial Methionine level ≥ 2 mg/dl - < 4 mg/dl
3. Maple syrup urine disease: Initial Leucine level ≥ 4 mg/dl - < 6 mg/dl
4. Galactosemia
    a) Beutler abnormal (no enzyme activity); Hill 10 - 14 mg/dl
    b) Beutler within normal limits (enzyme activity present); Hill 10 - 14 mg/dl
5. Biotinidase deficiency: partial to no enzyme activity
6. Hypothyroidism
    a) T4 < 5.5 mcg/dL
    b) TSH = 25 - 59 μU/ml
7. Hemoglobinopathies
    a) Hemoglobin Traits- F/A/S, F/A/C, F/A/D, F/A/E or FAB
    b) (A/F)- Adult hemoglobin present in higher quantity than fetal hemoglobin in an infant that has been transfused.
    c) (F/A/V)- Where "V" indicates the presence of an identified hemoglobin variant.
    d) Other- FD SD Disease (F/S/D)
8. Congenital adrenal hyperplasia

Birth weight category	Abnormal 17-OHP (ng/ml)
< 1250 gms	135-159
1250-1749 gms	90-134
1750-2249 gms	65-89
≥ 2250 gms	50-89

9. Medium Chain Acyl CoA Dehydrogenase Deficiency: Initial octanoylcarnitine level > or equal to 0.5 μmol/l to < 1.0 μmol/l.

C. Critical (or Clinically significant) Results:

1. Phenylketonuria: Initial Phenylalanine level ≥ 10 mg/dl

2. Homocystinuria: Initial Methionine level ≥ 4 mg/dl

3. Maple syrup urine disease: Initial Leucine level ≥ 6 mg/dl

4. Galactosemia

   a) Beutler abnormal (no enzyme activity); Hill ≥ 15 mg/dl

   b) Beutler within normal limits (enzyme activity present); Hill≥ 15mg/dl

   c) Three consecutive samples demonstrating abnormal Beutlers.

5. Biotinidase deficiency: Two consecutive samples demonstrating abnormal results. (Partial or no enzyme activity.)

6. Hypothyroidism

   a) TSH = 60 μU/ml or greater. (With Low or Normal T4 levels.)

   b) Two consecutive samples with low T4 levels

7. Hemoglobinopathies

   a) Sickle Cell Disease (F/S)
   b) C Disease (F/C)
   c) SC Disease (F/S/C)
   d) SE Disease (F/S/E)
   e) SD Disease (F/S/D)
   f) FE Disease (F/E)
   g) Other Hemoglobinopathies - Thalassemia (F/S/A)
   h) F Hgb only

8. Congenital adrenal hyperplasia

Birth weight category	Critical 17-OHP
< 1250 gms	≥ 160
1250-1749 gms	≥ 135
1750-2249 gms	≥ 90
≥ 2250 gms	≥ 90

9. Medium Chain Acyl CoA Dehydrogenase Deficiency: initial octanoylcarnitine level of > or equal to 1.0 μmol/l.

APPENDIX B

Unsatisfactory Specimen Criteria
Division of Consolidated Laboratory Services
Virginia Newborn Screening Laboratory, Richmond, Virginia

UNSATISFACTORY

The following unsatisfactory specimen criteria has been established in accordance with the standards provided by the National Committee for Clinical Laboratory Standards in regards to blood collection on filter paper for Neonatal Screening Programs (Document LA4-A3 Vol. 17 No. 16).

Specimens are reported as unsatisfactory according to the following descriptions. Refer to the returned portion of the sample card to identify the appropriate UNSAT code number.

UNSAT CODE NUMBER:

1. **SPECIMEN UNSAT - IMPROPERLY COLLECTED.**
   Specimens, which are not completely saturated when viewed from the reverse side or where the saturated blood does not completely fill the circle, or quantity insufficient for testing.

2. **SPECIMEN UNSAT - SCRATCHED OR ABRADED.**
   Specimen appears scratched, abraded, punctured, or indented due to applying blood with a capillary tube or other device.

3. **SPECIMEN UNSAT - WET.**
   Specimens which are wet or mailed prior to drying for a minimum of four hours.

4. **SPECIMEN UNSAT - OVER SATURATED.**
   Specimen appears streaked with blood clots, blood applied to both sides of filter paper, or layered with concentric circles of blood indicating multiple applications.

5. **SPECIMEN UNSAT - CONTAMINATED.**
   Specimen exhibits serum rings, or appears diluted, discolored, contaminated by antiseptic solutions, formulas, water, tissue fluids, or direct heat exposure.

6. **SPECIMEN UNSAT - NO BLOOD.**
   Filter spot is blank, failure to obtain blood specimen.

7. **SPECIMEN UNSAT - INSUFFICIENT INFORMATION.**
   Essential information for identification, categorization, interpretation and follow-up were not provided. Essential information includes last name, submitter(s), birth date, mother's name and date of specimen collection.

8. **SPECIMEN UNSAT - OLD SAMPLE > 10 DAYS IN TRANSIT.**
   The integrity of the sample may be compromised on specimen received after more than 10 days in transit. Analyses such as enzymes measured in newborn screening are heat labile and may be adversely affected by exposure of specimens to hot and/or humid environments.

9. **SPECIMEN UNSAT - INFANT > 6 MONTHS OLD.**
   Virginia newborn screening procedures and cut-off levels are based on the normal infant hematocrit. Samples from infants over six months of age are considered unsatisfactory for our screening procedures.

10. **SPECIMEN UNSAT - OUTDATED FILTER PAPER CARD.**
    Specimens collected on outdated filter paper cards may be compromised due to the unreliability of the filter paper. The age and condition of the filter paper directly affect absorption of blood.

11. **SPECIMEN UNSAT - INSUFFICIENT QUANTITY.**
    When testing has begun on a specimen and there is not enough specimen to complete each of the tests, some tests may be reported as unsatisfactory due to insufficient quantity.

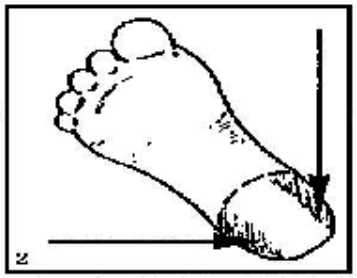
12. **SPECIMEN UNSAT - INTERFERING SUBSTANCES PRESENT.**
    Valid results could not be obtained due to interfering substances, e.g. antibiotics, anticoagulants, etc.

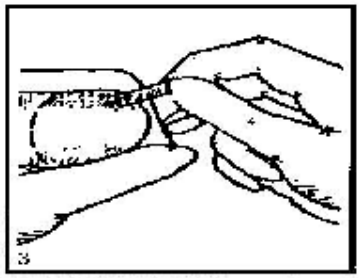
13. **SPECIMEN UNSAT – "OTHER".**
    This code number is reserved for any unsatisfactory specimen condition not appropriately described in codes 1 - 1 2. The NBS lab will provide comments to explain any unsatisfactory condition of this nature.

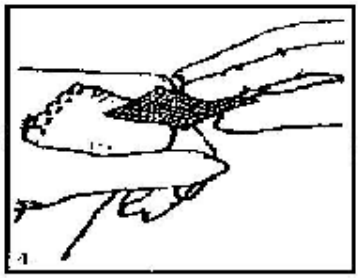
14. **SPECIMEN UNSAT - PARENTAL REFUSAL.**
    Hospital notification indicates that the baby's parents refused newborn screening at time of discharge.

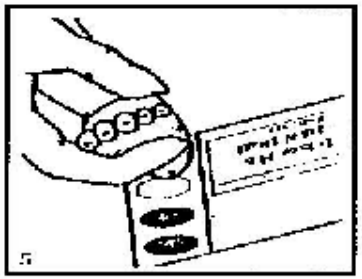
Note: Please obtain a valid specimen according to NCCLS guidelines, publication LA4-A "Blood Collection on Filter Paper for Neonatal Screening Programs". Allow a sufficient quantity of blood to soak through to completely fill the pre-printed circles with blood.Do not layer successive drops of blood or apply blood more than once in the same collection circle. Avoid touching or smearing spots. **For more information, please call the Virginia Newborn Screening Laboratory at (804) 648-4480 or toll free at 1-866-378-7730.**

Newborn Screening Specimen Collection









Collection Technique

- Using gloved hands cleanse infant's heel with 70% isopropyl alcohol (use only rubbing alcohol). see example 1.
- Allow heel to air dry. The puncture should be made within the shaded area in the drawing above. See example 2.
- Using lancet perform puncture as illustrated. See example 3.
- Gently wipe off first drop of blood with sterile gauze. (Initial drop contains tissue fluids which
- Wait for spontaneous flow of blood.
- Apply gentle pressure with thumb and squeeze intermittently as drops of blood form.
- Touch printed side of filter paper card to the blood drop and fill each printed circle with a SINGLE application of blood. Observe the saturation of each printed circle from reverse side as the blood flows through the filter paper insuring complete saturation. SPOTTING SHOULD BE DONE ONLY FROM THE FACE SIDE. See example 5
- Allow blood specimen to AIR DRY thoroughly, on level non-absorbant open surface, such as wire mesh, FOR AT LEAST TWO HOURS.
- Insure that collection form is completely filled out. Place dried specimen in an envelope and mail to the screening laboratory within 24 hours from the time of collection.

Problem Areas

- **Failing to wipe off alcohol residue may dilute the specimen and adversely affect test results.**
- **Puncturing the heel posterior to curvature causes blood to flow away from puncture, making proper collection difficult. DO NOT LANCE ON PREVIOUS PUNCTURE.**
- **Milking or squeezing the puncture may cause hemolysis and a mixture of tissue fluids with specimens.**
- **Layering successive drops of blood (see sample A) can cause uneven saturation. If blood flow diminishes to incompletely fill**

NOTE: See example B for unacceptable specimen with inadequate blood.

- Although not the preferred method, application of blood collected in sterile heparinized capillary tubes into the preprinted circles of the filter paper is an acceptable alternative.

1. Touch the tip of the heparinized capillary tube to the blood drop formed at the heel puncture site.
2. Collect approximately 100 microliters of blood into the heparinized capillary tube. USE A FRESH CAPILLARY TUBE FOR EACH BLOOD SPOT.
3. Apply the blood to the filter paper in one smooth continuous step without dabbing or other action that might scratch, compress or indent the paper. Waiting too long before application will allow cells and plasma to separate.

- Touching blood spots with latex exam gloves after collection on filter paper can cause contamination. Do not allow water, feeding formulas, antiseptic solutions, etc. to come in contact with the sample.
- Mailing specimens while still damp result in IMPROPER FILTER UNSATISFACTORY RESULTS.